Lasers in Surgery and Medicine 21:221–226 (1997)

RAPID REPORT

Irradiation of Infected Root Canals With a Diode Laser In Vivo: Results of Microbiological Examinations

A. Moritz, MD, DMD,[1]* N. Gutknecht, DMD,[2] U. Schoop, MD,[1]
K. Goharkhay, MD,[1] O. Doertbudak, MD,[1] and W. Sperr, MD, DMD[1]

[1]Department of Conservative Dentistry, Dental School, University of Vienna, Waehringerstrasse 25a, A-1090 Vienna, Austria
[2]Clinic of Conservative Dentistry and Periodontology, Aachen, Pauwelstrasse 30, D-52074 Aachen, Germany

Background and Objective: **It was shown in previous studies [1] that the Nd:YAG laser can be used as an excellent tool for killing bacteria in root canals. The present examinations were carried out with a high power diode laser in comparison with a conventionally treated control group.**
Study Design/Materials and Methods: **In this in vivo study, 40 patients with infected root canals underwent diode laser treatment. To verify the findings, microbiological tests were performed and the results compared to those obtained with conventional antibacterial treatment.**
Results: **The microbiological examination revealed streptococci at relevant concentrations in 20 cases and staphylococci in 5 cases. Extensive bacterial reduction was achieved in all cases by repeating laser treatment only once. Following the first irradiation, minimal streptococcal growth was observed in 7 root canals and minimal staphylococcal growth in 2 root canals. The maximum log kill was 4.22 for streptococci and 3.33 for staphylococci.**
In the control group, a maximum reduction by only one log step could be achieved in 6 of 10 patients.
Conclusions: **Compared with the results achieved with the conventional bactericidal technique in the control group, the high power diode laser seems to be highly suitable for killing bacteria in infected root canals. Lasers Surg. Med. 21:221–226, 1997.**

Key words: dentin; disinfection; endodontics; teeth

INTRODUCTION

It has always been a major goal of endodontic treatment to achieve a bacteria-free environment in the root canal to prevent any risk to successful root treatment. Normally, this is done with proteolytic, disinfecting irrigating solutions. With these solutions, infected and necrotic pulpal tissue is removed in toto and the root canal disinfected, enlarged and given a shape that allows optimal root canal filling. However, sterility of the root canal cannot be accomplished, [2] because microorganisms in the lateral canals and dentinal tubules can be removed neither instrumentally nor by irrigation with disinfecting solutions because of the small diameter of the tubules.

Electron microscopic examinations by Sen et

*Correspondence to: A. Moritz, DMD, Department of Conservative Dentistry, Dental School, University of Vienna, Waehringerstrasse 25a, A-1090, Vienna, Austria.

Accepted 27 May 1997

al. [3] revealed that penetration of bacteria into the dentinal tubules ranged from 10–150 μm. Moreover, some of the examined root canals were invaded by yeasts.

Vahdaty et al. [4] carried out in vitro tests with Enterococcus faecalis and found that chlorhexidine gluconate and NaOCl were equally effective antibacterial agents at similar concentrations. Although a marked reduction in the number of bacteria was achieved, 50% of the dentin samples remained infected.

Cavalleri et al. [5] succeeded in killing a large number of mainly gram-negative bacteria by repeated root canal irrigation with NaOCl. However, gram-positive bacteria, such as *Streptococcus lactis* and *Aerococcus*, persisted, probably because of environmental conditions such as acid pH.

Several studies have shown the favourable effects of laser treatment in this area. Encouraging results were obtained with the CO_2 laser [6,7], the Nd:YAG laser [8,9,10,11,12] and the Excimer laser [13,14]. In an in vitro study, we examined the effects of diode laser irradiation on infected root canals and obtained promising results [15].

With the great progress in the field of laser technology, semiconductor lasers are gaining increasing importance. Approximately ten years ago, the available output ranged around 100 mW, whereas, today, some laser diodes can achieve an output power of several W. Semiconductor lasers have shown to be highly reliable and effective. The diode laser today represents 99% in volume and 25% in value of all lasers sold [16]. By combining several laser diodes into larger arrays, the output power can be increased up to the kilowatt level, which was formerly a domain of gas and solid lasers.

Wyman et al. [17] reported initial experience of using a 805 nm/10W diode laser prototype to incise and vaporize human uterus and colon in vitro and rat liver in vivo. Results showed great similarity between the diode and Nd:YAG laser when used in contact mode. Subsequent experiences with a 25W clinical system confirmed the initial results [18].

In a theoretical paper on the equivalency of a 810 nm diode laser and the Nd:YAG laser for prostate coagulation, Rastegar et al. [19] stated that Nd:YAG laser radiation has a greater penetration depth in prostate and myocardial tissues than diode laser radiation. However, the latter caused a greater increase in temperature at the irradiated surface.

Millard et al. [20] stated that the Nd:YAG laser and the 805 nm diode laser are de facto equally effective in contact mode and can be used as alternatives in medicine. So far, it seems that only very few diode lasers are being used in the field of dentistry.

After having used the Nd:YAG laser in clinical routine for several years, in this study, we aimed to examine the diode laser's antimicrobial abilities within the scope of endodontic therapy.

MATERIALS AND METHODS

In the present study, a total of 40 patients (all of them had given their informed consent in taking part in our study) with infected root canals were treated. Each patient underwent four sessions of combined laser treatment and microbiological examinations. Only single-rooted teeth (upper and lower incisors and single rooted premolars) took part in this study. All the teeth had been devital prior to treatment (negative reaction in the vitality-test using frozen CO_2 as a stimulus) and showed radiographical signs of an apical inflammation. After the application of a rubber dam, the crowns were disinfected and dried; then the infected root canals were prepared mechanically using the step-back-technique up to a width of 0.5mm irrigating only with physiologic NaCl solution.

To determine the initial values, a first series of microbiologic samples were collected prior to laser treatment. For this purpose, the root canal was irrigated with 0.5 ml physiologic saline solution and one sterile paper point of a defined size (size 40 point) introduction into the root canal for 10 seconds. The paper point was put into brain-heart infusion (BHI) and inactivating agent (9% Tween 80 + 0.9% lecithin + 0.3% histidin + 0.5% sodium thiosulfate) immediately after being removed from the root canal and extracted for 30 seconds to prepare the sample for the subsequent microbiologic examination.

This procedure was followed by the actual laser treatment. The root canals were irradiated with a 810 nm diode laser by Dentek Laser Systems, Gaisfeld, Austria. The laser output power ranges from 0.5–15 Watt. A pulse rate of 2–32 ms in pulsed mode and a frequency of 1.5Hz–250 Hz can be used. The laser can also be operated in continous wave (cw) mode. The target beam is generated by a He/Ne laser (533 nm, 1mW). For root canal treatment, we used a setting of 2 W at a pulse rate of 20 ms, 50 Hz.

The laser beam is transferred to the handpiece via a flexible glass fiber; in the handpiece it is coupled to the actual application-tip with an outer diameter of 400μm. The handpiece and the application-tip were sterilized after each individual treatment.

The optical fiber was inserted as far as the apex; the correct insertion depth was ensured by measuring. The laser was then activated and the root canal slowly irradiated from apical to coronal in continuous circling movements to treat all dentinal tubules. Irradiation was repreated 5 times at each laser treatment, each time for a period of 5 seconds with short breaks in-between.

Following irradiation, the tooth crown was cleaned with H_2O_2 and the exposed root canal sealed with a sterile cotton pellet and zinc oxyphosphate cement. The second series of microbiologic samples was collected after four days. After application of a rubber dam, the crown was cleaned with H_2O_2 and the zinc oxyphosphate cement removed with a sterile rose-head bur. The cotton pellet was removed as well. As described previously, the root canal was irrigated with 0.5 ml physiologic saline solution, samples were obtained with a sterile paper point for 10 seconds and the paper points put into the transport medium. After the samples had been collected, the root canals were lased for a second time, irrespective of whether the first irradiation had resulted in sufficient bacterial reduction or not. The root canals were again sealed with zinc oxyphosphate cement and a cotton pellet.

At the third session after another four days, we collected the final microbiologic samples. The final filling of the root canals was carried out at the fourth session.

The microbiologic examinations as mentioned above were performed using sterile paper points which were put into BHI and inactivating agent and extracted for 30 seconds.

Two ml extracted fluid were diluted in log 10 steps with BHI + inactivating agent. 0.1 ml portions of the dilutions to 10^{-1}–10^{-5} were applied on Columbia agar plates and HLR agar plates with a spatula [21]. The plates were incubated aerobically for 24 hours at 37°C. The colonies were then counted and the total number of bacteria (colony-forming units = CFU) per ml of the extracted fluid assessed. Catalase activity was tested by adding a few drops of H_2O_2 to some colonies.

In addition, a control group consisting of 10 patients was treated just the same way as those of the laser group, except for the actual application of the laser. Instead of rinsing the root canals with a NaCl solution, a 3% hydrogen peroxide solution was used.

RESULTS

The microbiological examination of the samples, which had been collected prior to the first laser irradiation (initial values), revealed streptococci in 40 cases and staphylococci in 5 cases.

After the first irradiation, only minimal streptococcal growth was observed in 7 root canals and minimal staphylococcal growth in 3 root canals. However, already the *initial* bacterial counts of twenty root canals with streptococci had been so low (log CFU/ml = 1.00) that no significant statement about bacterial reduction was possible in these cases.

The residual 13 samples with streptococci and 2 samples with staphylococci, which had shown bacterial growth after the first irradiation, showed minimal bacterial growth (log CFU/ml = 1.00) after the second irradiation (value 2).

The bacterial count ranged between 10^1 and 8.5×10^5 for all bacteria. The maximum logarithmic reduction factor was 4.22 for streptococci and 3.33 for staphylococci. We found no correlation between the bacterial count and the required number of irradiations.

The following tables give an overview of the results.

Initial and post-irradiation values (after 4 days = PIV1, after 8 days = PIV2) of bacterial counts (log CFU/ml) and log reduction factors (after 4 days = RF1, after 8 days = RF2).

A log value of 1 corresponds to minimal bacterial counts (1–9 colonies). Only cases with a significant initial value are mentioned in the tables.

In the control group, streptococci were found in all patients at relatively low initial bacterial counts ranging around 5.0×10^3. Staphylococci were found in 3 patients. The maximum logarithmic reduction factor was 1.37 for streptococci and 1 for staphylococci. The repetition of the treatment did not show any significant improvement.

DISCUSSION

Cleaning, disinfection, and preparation of the root canal are indispensable requirements for successful endodontic treatment. Therefore, attempts have been made to combine the mechani-

TABLE 1. Streptococci

		log CFU/ml		log RF	
Patient	IV	PIV1	PIV2	RF1	RF2
1	5.33	1.12	1.00	4.21	4.33
2	3.32	1.47	1.00	1.85	2.32
3	5.14	2.10	1.47	3.04	3.67
4	3.45	1.00	—	2.45	—
5	5.76	2.77	1.00	2.99	4.76
6	5.42	1.20	1.00	4.22	4.42
7	4.70	2.12	1.00	2.58	3.7
8	5.20	1.27	1.00	3.93	4.2
9	5.84	3.10	1.45	2.74	4.39
10	4.44	2.2	1.00	2.24	3.44
11	3.10	1.00	—	2.1	—
12	3.20	1.00	—	2.2	—
13	5.47	2.2	1.00	3.27	4.47
14	3.74	1.00	—	3.74	—
15	3.46	1.00	—	2.46	—
16	3.70	1.00	—	2.70	—
17	3.77	2.1	—	1.67	—
18	5.90	2.74	1.00	3.16	4.9
19	3.26	1.00	—	2.26	—
20	5.6	2.1	1.00	3.5	4.6

Staphylococci

		log CFU/ml		log RF	
Patient	IV	PIV1	PIV2	RF1	RF2
3	4.33	1.00	—	3.33	—
5	3.2	1.00	—	3.2	—
11	3.1	1.77	1.00	1.33	2.1
17	3.62	1.00	—	2.62	—
20	4.2	2.1	1.00	2.1	3.2

TABLE 2. Streptococci (Control Group)

		log CFU/ml		log RF	
Patient	IV	PIV1	PIV2	RF1	RF2
1	3.18	1.93	1.73	1.25	1.45
2	3.44	2.07	1.98	1.37	1.46
3	3.24	2.14	2.28	1.10	0.96
4	4.33	4.1	2.88	0.23	0.45
5	3.6	2.37	2.44	1.23	1.16
6	3.1	3.0	2.8	0.1	0.3
7	4.2	3.55	3.2	0.65	1
8	3.12	2.17	2.1	0.95	1.02
9	3.94	3.22	3.2	0.72	0.74
10	3.33	2.1	2.66	1.23	0.77

Staphylococci (Control Group)

		log CFU/ml		log RF	
Patient	IV	PIV1	PIV2	RF1	RF2
3	3.3	2.4	2.1	0.9	1.2
7	3.67	2.8	2.72	0.87	0.95
10	3.34	2.34	2.44	1	0.90

cal preparation of root canals with the irrigation with proteolytic, disinfecting solutions to achieve optimal sterilization of the root canal environment prior to root filling [2,22]. In consequence, many attempts (as already cited in the introduction section) have been made to use the sterilizing power of the laser beam, and in fact, mostly encouraging results were obtained.

Hardee et al. [23] irradiated root canals of extracted teeth with the Nd:YAG laser at an output power of 3 W for 1–2 minutes or 180–360 J. Their test bacterium, *Bacillus stearothermophilus*, is a highly heat-resistant, sporiferous bacterium. They achieved a bacterial reduction by 99%, corresponding to a log kill of 2.

In an experiment in animals, Bahcall et al. [24] examined the histological effect of laser irradiation at an output power of 3 W for 30 seconds on periradicular tissue and observed ankylosis, lysis of cementum, and bone transformations after 30 days.

Tseng et al. [25] found that irradiation with a Nd:YAG laser had a bactericidal effect on bacterial plaque on root surfaces. No bacteria were left after the roots had been irradiated at an output power of 1.75 W for 30 seconds. However, the authors carried out no qualitative analysis of their results.

White et al. [26] succeeded in reducing *Bacillus subtilis* and *Escherichia coli* by log 6 through irradiation with a Nd:YAG laser at 60 J and more.

In a laboratory experiment, Rooney et al. [27] achieved a 1000-fold reduction in heat-resistant bacteria (*Enterococcus faecalis*) through irradiation with a pulsed Nd:YAG laser, corresponding to a log kill of 4. They required an energy of 1.8 W for 30 seconds or 54 J, respectively. When black Suomi ink was used as absorbent, the same result was achieved at 25 J.

Gutknecht et al. [1] examined the bactericidal effect of a pulsed Nd:YAG laser at standard settings (1.5 W, 40 s, or 60 mJ) in classically prepared root canals in vitro. They were able to eliminate 99.91% of the injected *Enterococcus faecalis* bacteria on average. This corresponds to a log kill of 3.07. The maximum and minimum log kills were 5.46 and 1.54, respectively.

As opposed to most preceding investigators who performed in vitro studies or animal tests, we tried to assess the effects of root canal laser treatment under in vivo conditions in humans.

In our treatment of root canals with the diode laser, a maximum of two irradiations resulted in nearly complete elimination of bacteria. This goal was reached through only one irradiation in more than 50% of the cases. The maximum log kill was 4.22 for streptococci and 3.33 for staphy-

lococci. In the control group, though an antibacterial rinsing solution was used, only a limited reduction of bacteria (by one log step) could be achieved.

In vitro examinations revealed, that the risks of thermal side effects during endodontic diode laser treatment are very low. An irradiation at a laser output power of 2 W/50 Hz for 25 seconds with breaks in-between, suffices for sterilization of the root canal and is within the lower range from an international point of view. The relatively long duration of irradiation and appropriate guidance of the light conductor fiber in conjunction with the enlargement of the beam diameter in a short distance from the outlet guarantees sufficient treatment of the entire root canal wall and irradiation of all bacteria.

In view of the very encouraging results of this study, the diode laser can be considered equal to the Nd:YAG laser in endodontic treatment. The use of appropriate absorbents for optimisation of the laser effect would surely be of great interest: studies investigating this subject are being carried out at the moment.

In 1994, McKinley and Ludlow evaluated the potential for spreading bacterial contamination from the root canal to the patient and the dental team via the smoke produced by the laser [28]. In the smoke plume of an argon laser, viable bacteria have been found. Although during our investigations using the diode laser, no formation of visible smoke could be observed; however, it cannot be completely excluded that viable bacteria can be spread to the environment during the laser procedure.

In 1990, Ando and Hoshino examined the presence of anaerobic vs aerobic microorganisms in the root canal dentine; they stated, like Zielke et al. in 1976, that anaerobic microorganisms are predominant especially in deep layers of infected root dentine [29,30]. Our study confined itself on aerobic culturing; however, these aerobic microorganisms were used as an indicator for the antimicrobial effects of diode laser irradiation. In our opinion, it can be assumed, that the effects on anaerobic microorganisms is thoroughly comparable. However, a complementary study including anaerobes could be a future project.

Another study is currently being carried out to evaluate and compare clinical and radiographic criteria of complete healing in the case of focal infection.

REFERENCES

1. Gutknecht N, Moritz A, Conrads G, Sievert T, Sperr W, Lampert F. Bactericidal Effect of the Nd:YAG Laser *in Vitro* Root Canals. J Clin Las Med Surg 1996; 14/2: 77–80.
2. Guldener PHA, Langeland K. Aufbereitung und Reinigung des Wuzrzelkanals. in *Chapter 13* "Endodontologie-Diagnostik und Therapie." Thieme, Stuttgart/New York 1992.
3. Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. Endod Dent Traumatol 1995; 11:6–9.
4. Vahdaty A, Pitt Ford TR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules in vitro. Endod Dent Traumatol 1993; 9:243–248.
5. Cavalleri G, Cuccolin L, Urbani G, Benoni G. Root Canal Microflora: Qualitative Changes after Endodontic Instrumentation. J Chemotherapy 1989; 1(2):101–1022.
6. Moritz A, Schoop U, Nell A, Wernisch J, Sperr W. Veränderungen der Wurzelkanaloberfläche unter Bestrahlung mit dem CO_2 Laser. Ergebnisse einer in-vitro-Studie. Z Stomatol 1995; 92/7:343–348.
7. Wilkert Walter C. Bakterizide Wirkung des CO_2-Lasers im Wurzelkanal-eine in vitro Studie. 6. Internationaler Jahreskongreβ der Deutschen Gesellschaft für Laserzahnheilkunde, Frankfurt/Main, Germany, 31. 1–02.02. 1997.
8. Gutknecht N, Behrens VG. Die Bearbeitung der Wurzelkanalwände mit dem ND:YAG-Laser. Zahnärztl Welt 1991; 100:748–755.
9. Gutknecht N, Behrens VG. The Nd:YAG laser as an aid to root canal obturation. 79th Annual World Dental Congress of FDI 1991.
10. Goodis HE, White JM, Marshall SJ, Marshall GW Jr. Scanning electron microscopic examination of intracanal wall dentin: Hand vs laser treatment. Scanning-Microsc 1993; 7(3):979–987.
11. Hardee MW, Miserendino L, Kos W. Evaluation of the antibacterial effects of intracanal Nd:YAG laser irradiation. 47th Annual Session American Association of Endodontics, 1990, Las Vegas Nevada.
12. Bahcall J, Howard DVM, Miserendino L, Walis H. Preliminary investigation of the histological effects of laser endodontic treatment on the periradicular tissues in dogs. J Endod 1992; 18:47–51.
13. Liesenhoff F, Lenz H, Seiler T. Wurzelkanalaufbereitung mit Excimer Laserstrahlen. Zahnärztl Welt 1989; 98: 1034.
14. Frentzen M, Koort HJ, Nolden R. Aufbereitung von Wurzelkanälen mit Excimer Lasern. Dtsch Zahnärztl Z 1991; 46:288–289.
15. Moritz A, Gutknecht N, Goharkhay K, Schoop U, Wernisch J, Sperr, W. In vitro irradiation of infected root canals with a diode laser: Results of microbiologic, infrared spectrometric, and stain penetration examinations. Quintessence International 1997; 28/3:205–209.
16. Kales D. Review and Forecast of Laser Markets 1993. Laser Focus World 1993; 29:72–73.
17. Wyman AL, Duffy S, Sweetland HM, Sharp F, Rogers K. Preliminary evaluation of a new high power laser. European Laser Association Meeting, Graz, Austria (Nov. 1990); Lasers Surg Med 1992; 12:506–509.
18. Wyman AL, Duffy S, Sweetland HM, Sharp F, Rogers K.

Laser tissue interactions of diode lasers at 805 nm (1992) Lasers Surg Med Suppl 4:1992; 384–385.

19. Rastegar S, Jacques SL, Motamedi M, Kim BM. Theoretical Analysis of Equivalency of High-Power Diode Laser (810nm) and Nd:YAG Laser (1064) for Coagulation of Tissue: Predictions for Prostate Coagulation. SPIE Proceedings: Laser-Tissue Interaction III, 1992; 1646:150–160.
20. Millard MJ, Matthews L, Aronoff BL, Hults D. Soft Tissue Studies With 805 nm Diode Laser Radiation: Thermal Effects With Contact Tips and Comparison With Effects of 1064 nm Nd:YAG Laser Radiation. Lasers Surg Med 1993; 13:528–536.
21. Ritz HL. Microbial population shifts in developing human dental plaque. Archs Oral Biol 1967; 12:1561–1568.
22. Petschelt A. Endodontie: Maschinell-manuell? Die Aufbereitung und Spülung des Wurzelkanals. Dtsch Zahnärztl Z 1989; 44:407.
23. Hardee MW, Miserendino L, Kos W. Evaluation of the antibacterial effects of intracanal Nd:YAG laser irradiation. 47th Annual Session American Association of Endodontics, 1990, Las Vegas Nevada.
24. Bahcall J, Howard DVM, Miserendino L, Walis H. Preliminary investigation of the histological effects of laser endodontic treatment on the periradicular tissues in dogs. J Endod 1992; 18:47–51.
25. Tseng P, Gilkeson CF, Palmer J, Liew V. The bacteriocidal effect of a Nd:YAG laser in vitro. Dent Res 1991; 70:650.
26. White JM, Goodis HE, Cohen JN. Bacterial reduction of contaminated dentine by Nd:YAG laser. J Dent Res 1991; 70:412 (abstract 1170).
27. Rooney J, Midda M, Leeming J. A laboratory investigation of the bactericidal effect of a Nd:YAG laser. Br Dent J 1994; 176:61.
28. McKinley IB, Ludlow MO. Hazards of laser smoke during endontic therapy. J Endod 1994; 20(11):558–559.
29. Ando N, Hoshino E. Predominant obligate anaerobes invading the deep layers of root canal dentine. Int Endod J 1990; 23:20–27.
30. Zielke DR, Heggers JP, Harrison JW. A statistical analysis of anaerobic versus aerobic culturing in endodontic therapy. Oral Surgery, Oral Medicine and Oral Pathology 1976; 42:830–837.